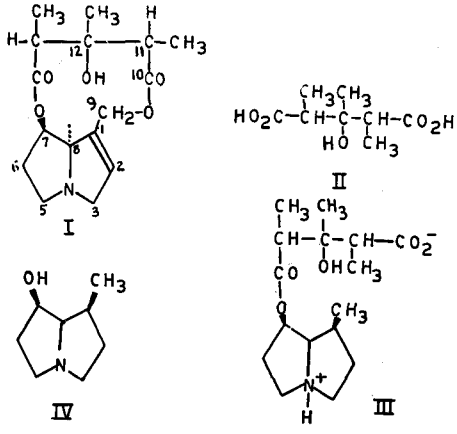
Genus *Crotalaria* XXI[1]. Isocromadurine, a Novel Pyrrolizidine Alkaloid of *C. madurensis* R. Wight

The seeds of *C. madurensis* have been reported[1-3] to contain 4 alkaloids: namely, cromadurine, madurensine, fulvine and crispatine. In this communication we wish to record the isolation and structure of a new macrocyclic pyrrolizidine alkaloid isocromadurine (I).

Alcoholic extract of the defatted seeds (containing 1.9% alkaloids) was processed to give alkaloid mixture, which on crystallization from methanol-acetone yielded cromadurine followed by fulvine. The mother liquor, after removal of cromadurine and fulvine, was evaporated to dryness. The residue was crystallized from petroleum ether to give colourless crystals of isocromadurine, m.p. 135–136° $(\alpha)_D^{15}$ + 43.54 (C, 0.85%, ethanol), TLC (silica gel G treated with N/10 NaOH, methanol) showed single spot



of Rf 0.22 (cf. monocrotaline 0.22). The elemental analyses of isocromadurine and its picrate (m.p. 204–205°) conform to the molecular formula $C_{16}H_{23}O_5N$, M+309. The IR-spectrum (KBr) of the base exhibited peaks due to ester carbonyls (1730 cm^{-1}) and –OH group (3400 cm^{-1}).

The NMR-spectrum (60 MHz, $CDCl_3$) of isocromadurine exhibits signals at δ 1.17 (s, CH_3–C–OH), 1.27 (d, J 7.5 Hz, 2 × CH_3–CH), 2.00 (m, H 6), 4.60 (ABq, J 12 Hz, H 9) and 5.97 (m, H 2). The presence of –OH group at δ 4.50 was shown by D_2O exchange. In the MS (M+ 309) of isocromadurine, the peaks at *m/e* 80, 93, 95, 119, 120, 121, 136 and 138 suggested it to be an ester of retronecine type amino alcohol. The location of –OH group at C–12 is strongly supported[4] by the presence of ion *m/e* 236 (43%).

The base on hydrolysis with 2 *N* methanolic NaOH at room temperature gave retronecine and isocromaduric acid (II), $C_8H_{14}O_5$, m.p. 129–130° (ether- pet. ether), $(\alpha)_D^{32}$ + 14.9° (C, 0.91%, methanol). The NMR- and IR-spectra of isocromaduric acid were identical with those of cromaduric acid[1], m.p. 138–139°, $(\alpha)_D^{32°}$ – 14.5° (methanol).

When hydrogenated over reduced PtO_2 in ethanol, isocromadurine absorbed 2 moles of hydrogen to give the zwitter ion (III), m.p. 183–184° (ethanol-acetone), $\nu_{max}^{CHCl_3}$ 3550 (–OH), 1720 (CO), 1460 cm^{-1} (–N^+ H). The zwitter ion on hydrolysis[5] with 2 *N* sodium hydroxide in methanol yielded retronecanol (IV), picrate m.p. 207–208° and isocromaduric acid[6].

Zusammenfassung. Strukturaufklärung eines neuen Pyrrolizidinalkaloids, Isocromadurin, aus dem Samen von *Crotalaria madurensis* R. Wight. isoliert.

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[1] For part XX see: P. G. RAO, R. S. SAWHNEY and C. K. ATAL, Indian J. Chem. in press (1975).
[2] C. K. ATAL, K. K. KAPUR, C. C. J. CULVENOR and L. W. SMITH, Tetrahedron Lett. *1966*, 537.
[3] A. A. M. HABIB, M. R. I. SLEH and M. A. FARAG, Lloydia *34*, 455 (1971).
[4] L. B. BULL, C. C. J. CULVENOR and A. T. DICK, in *The Pyrrolizidine Alkaloids* (North-Holland Publishing Co., Amsterdam 1968), p. 56.
[5] S. C. PURI, R. S. SAWHNEY and C. K. ATAL, Experientia *29*, 390 (1973).
[6] We wish to thank Dr. NITYA ANAND, Director, C.D.R.I., Lucknow for IR-, NMR- and to Dr. B. D. TILAK, Director, NCL, Poona for the mass spectrum.

Sterols of the Lobster (*Homarus americanus*) and the Shrimp (*Pandalus borealis*)[1]

The diversity of sterols in marine invertebrates is of interest from the viewpoint of both chemotaxonomy and comparative biochemistry. There are, however, only a few reports on the sterol composition of marine crustacea, although this class consists of a great number of species and is a major contributor to the biomass of the oceans[2-6]. In this paper we report the isolation of two new sterols, 24-methylcholesterol and 24-ethylcholesterol, from two species of continental shelf crustacea, the lobster (*Homarus americanus*), and the shrimp (*Pandalus borealis*) and discuss possible sources of these two sterols.

Methods. Lobsters were collected in June, 1973, from Vineyard Sound, Massachusetts. Shrimp were collected from 42°43'N, 65°09'W using No. 41 bottom trawls and immediately frozen on the R/V ALBATROSS IV, Cruise 73–3 in May of 1973.

The live lobsters and frozen shrimp were extracted for sterols by a modified procedure of KRITCHEVSKY[4], and IDLER and WISEMAN[5]. Further purification of the sterols was accomplished by formation of their digitonide derivatives[7]. After cleavage of the sterol digitonides to the free sterols and conversion of the sterols to their respective trimethylsilylated derivatives, gas chromatographic analyses were performed[8].

[1] Woods Hole Oceanographic Institution Contribution Number 3494.
[2] J. AUSTIN, in *Advances in Steroid Biochemistry and Pharmacology* (Ed. M. H. BRIGGS; Academic Press, New York, N. Y. 1970), p. 73.
[3] S. TESHIMA, Mem. Fac. Fish., Kagoshima Univ. *21*, 69 (1972).
[4] D. KRITCHEVSKY, S. A. TEPPER, N. W. DITULLO and W. L. HOLMES, J. Food Sci. *32*, 64 (1967).
[5] D. R. IDLER and P. WISEMAN, Int. J. Biochem. *2*, 91 (1971).
[6] S. YASUDA, Comp. Biochem. Physiol. *44B*, 41 (1973).
[7] C. H. ISSIDORIDES, I. KITAGAWA and E. MOSETTIG, J. org. Chem. *27*, 1493 (1962).
[8] R. B. GAGOSIAN, Geochim. cosmochim. Acta, in press (1975).

Final structure proof for the trimethylsilylated derivatives was based on molecular ion and fragmentation patterns of authentic standards obtained by gas chromatography-mass spectrometry[8].

Results and discussion. The sterol content found was 0.075% for *Homarus americanus* and 0.13% for *Pandalus borealis*, based on wet weight (see Table I). Although *Pandalus* has approximately twice the lipid content of *Homarus*, the concentration of unsaponifiable matter for both is almost the same. Each entry of Tables I and II represents an average of 6 analyses. Approximately 30 shrimp and 3 lobsters were used for their respective individual analyses. When comparisons of female and male, non-molted and molted lobster extracts were made, no differences in sterol content were noted.

Sterol distributions and standard deviations for both *Homarus* and *Pandalus* are listed in Table II. Note the high cholesterol content in both species. It is consistent with previous results of other workers that cholesterol is the most abundant sterol in the advanced invertebrates. The more primitive invertebrates have much more diversified sterol compositions[2,5]. To the best of our knowledge, 24-methylcholesterol and 24-ethylcholesterol have not been reported previously for any species of marine decapod crustacea of the suborder *Macrura*. Desmosterol, 24-methylenecholesterol and 22-dehydrocholesterol, 3 sterols observed in our samples (Table II), are the only sterols reported in decapods other than cholesterol[4-6,9]. Fucosterol, a common brown algal sterol[2]; brassicasterol, a diatom sterol[10]; stigmasterol and norcholestadienol were not detected by our procedure. In addition no Δ^7 sterols were detected.

Both 24-methylcholesterol and 24-ethylcholesterol have alkyl groups at C-24; therefore, two C-24 epimers may be present, campesterol and 22,23-dihydrobrassicasterol for 24-methylcholesterol and β-sitosterol and clionasterol for 24-ethylcholesterol. However, the amounts isolated were too small for nuclear magnetic resonance (NMR) analysis and the stereochemical assignments will have to await further work.

Table I. Total lipid, unsaponifiable lipids and sterol content in *Homarus americanus* and *Pandalus borealis*

	Percentage of wet weight of tissue		
	Total lipids	Unsaponifiable lipids	Sterols
Homarus americanus	0.66 ± 0.02	0.62 ± 0.03	0.08 ± 0.008
Pandalus borealis	1.24 ± 0.06	0.66 ± 0.03	0.13 ± 0.005

Table II. Percentage composition of *Homarus americanus* and *Pandalus borealis* Sterols

Compound[a]	*Homarus americanus*	*Pandalus borealis*
Cholesterol	97.8 ± 0.3	94.3 ± 0.6
Desmosterol	0.2 ± 0.1	4.2 ± 0.4
24-Methylcholesterol	0.6 ± 0.2	0.6 ± 0.2
24-Ethylcholesterol	0.7 ± 0.2	0.5 ± 0.2
24-Methylenecholesterol	0.3 ± 0.1	0.4 ± 0.2
22-Dehydrocholesterol	0.4 ± 0.2	trace

[a] Cholesterol: cholest-5-en-3β-ol; desmosterol: cholesta-5,24-dien-3β-ol; 24-methylcholesterol: 22,23-dihydrobrassicasterol or campesterol; 24-ethylcholesterol: β-sitosterol or clionasterol; 24-methylenecholesterol: ergosta-5,24(28)-dien-3β-ol; 22-dehydrocholesterol: cholesta-5,22-dien-3β-ol.

Sterol reduction products (cholestanol, campestanol and stigmastanol) are difficult to separate from their parent sterols (cholesterol, campesterol and β-sitosterol) by GC. In previous studies it is not clear whether the investigators searched for these compounds[4,6,9]. In the present study we did not detect any of these stanols as determined by mass chromatography.

Although the isolation and structure determination of sterols in crustacea have received a great deal of attention in recent years, very little work has been accomplished on the sources of these compounds in marine animals. Previous work has shown that most species of crustacea cannot biosynthesize cholesterol or other sterols from acetate or mevalonic acid through the squalene to lanosterol or cycloartenol pathway[11,12]. Selected crustaceans, e.g. lobster, shrimp and crab can, however, biosynthesize cholesterol from other sterols such as campesterol, β-sitosterol, ergosterol, and brassicasterol[3]. Cholesterol and these sterol precursors to cholesterol must, therefore, be acquired by crustacea from external sources in the form of food such as marine invertebrates, phytoplankton and fish or through absorption or filtration of sterols from the dissolved or particulate fraction of seawater[8].

Examination of the sterol distribution in the lobster's and shrimp's seawater environment revealed the presence of cholesterol and β-sitosterol as the major sterols with high concentrations of campesterol in early summer phytoplankton blooms[13]. Lower concentrations of 24-methylenecholesterol, 22-dehydrocholesterol, norcholestadienol, fucosterol, brassicasterol and stigmasterol were also found.

One of the major sources of campesterol in the marine environment appears to be marine yeasts[14]. In addition, a few species of phytoplankton[15,16], and molluscs[17,18] have been found to contain small quantities. Although ergosterol is the major sterol in marine yeasts and fungi, it was not detected in lobsters, shrimp or seawater. This sterol may, therefore, be metabolized rapidly to cholesterol or other organic compounds by crustacea or is possibly metabolized by microorganisms in seawater. β-Sitosterol is a major sterol in terrestrial plants. It may enter the marine environment through river runoff or aeolian transport on particulates. This sterol has also been found in coastal grasses in the Gulf of Mexico[19] and in low concentration in molluscs[17].

As mentioned above, lobsters and shrimp are able to convert campesterol and β-sitosterol to cholesterol; however, low concentrations of these two sterols still persist in these animals. Both these sterols are over 10^4 times higher in concentration in *Homarus* and *Pandalus*

[9] S. Teshima and A. Kanazawa, Bull. Jap. Soc. scient. Fish. *37*, 63 (1971).
[10] A. Kanazawa, M. Yoshioka and S. Teshima, Bull. Jap. Soc. scient. Fish. *37*, 899 (1971).
[11] S. Teshima and A. Kanazawa, Comp. Biochem. Physiol. *38B*, 597 (1971).
[12] D. I. Zandee, Comp. Biochem. Physiol. *20*, 811 (1967).
[13] R. B. Gagosian, unpublished results.
[14] S. Teshima and A. Kanazawa, Bull. Jap. Soc. scient. Fish. *37*, 68 (1971).
[15] J. Boutry and C. Baron, Bull. Soc. Chim. biol. *49*, 1399 (1967).
[16] J. Boutry and G. Jacques, Bull. Soc. Chim. biol. *52*, 349 (1970).
[17] D. R. Idler and P. Wiseman, Int. J. Biochem. *2*, 515 (1971).
[18] S. Teshima and A. Kanazawa, Bull. Jap. Soc. scient. Fish. *38*, 1299 (1972).
[19] D. H. Attaway, P. Haug and P. L. Parker, Lipids *6*, 687 (1971).

when compared with sterols isolated from the animal's seawater environment[13]. These crustacea, then, are not converting all the ingested campesterol and β-sitosterol to cholesterol, and a steady state concentration of these two compounds appears to exist in these organisms. More work, however, is clearly needed before this hypothesis is proven.

From our results, it appears that caution should be exercised when comparing sterol distributions among members of the same species sampled from different locations. The sterol content of the marine fauna and flora in the animal's surrounding oceanic environment should also be considered. The sterols present in the animal's seawater environment may be an indicator of this faunal and floral contribution to the overall sterol content of the animal[20].

Summary. In this study we have analyzed the sterol compositions of two continental shelf species of crustacea, the lobster (*Homarus americanus*) and the shrimp (*Pandalus borealis*). Cholesterol was found to be the most abundant sterol in these two species with smaller amounts of desmosterol, 24-methylcholesterol, 24-ethylcholesterol, 24-methylenecholesterol and 22-dehydrocholesterol.

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Piperaceae Alkaloids: Part II[1] Structure and Synthesis of Cyclostachine A, A Novel Alkaloid from *Piper trichostachyon* C. DC[2]

In a previous communication[2] we have assigned the structure (I) for piperstachine, isolated from the stem of *Piper trichostachyon* C. DC. We wish to report here the isolation and structure elucidation of a new alkaloid, cyclostachine A (II).

The alkaloid, isolated by cold percolation of the stems with hexane and chromatography over alumina had m.p. 136–138°. It analyzed for $C_{22}H_{27}NO_3$, and showed λ_{max}^{EtOH} 235, 287 nm (log ε 3.65, 3.62), ν_{max}^{KBr} 1630 cm^{-1} (tertiary amide). Its mass spectrum exhibited the molecular ion peak at *m/e* 353 and intense ions at *m/e* 255 and 98 due to the cleavage as shown in II and *m/e* 135 due to methylenedioxytropylium ion. The alkaloid is racemic as shown by CD and ORD determinations. In its ^{1}H-NMR-spectrum ($CDCl_3$, 100 MHz) cyclostachine A shows the following signals: δ (ppm from TMS) 6.7 (3H, m, $H_{2,5,6}$); 5.90 (2H, s, H_{22}); 5.92 (1H, d,d,d, $J_{9,8}$ = 10, $J_{9,10}$ = 5, $J_{9,7}$ = 2 Hz, H_9); 5.56 (1H, d(br), $J_{8,9}$ = 10 Hz, H_8); 3.68 (1H, d, q, $J_{7,16}$ = 10 Hz, H_7); 3.40 (2H, t, J = 6.5 Hz, H_{18} or H_{21}); 2.76 (1H, d,d, $J_{16,15}$ = 11, $J_{16,7}$ = 10 Hz, H_{16}); 3.0–3.3 (1H, m, H_{10}); 2.0–2.5 (3H, m, H_{15} and H_{21} or H_{18}); 1.0–2.0 (12H, m, $H_{11,12,13,14,19,20}$). The proton-noise decoupled ^{13}C-NMR-spectrum of the alkaloid ($CDCl_3$) shows 22 lines, and off-resonance partial decoupling experiments gave the multiplicity of each signal leading to the following assignments, δ (ppm from TMS): 173.6 (C_{17}), 147.7, 146.3 ($C_{3,4}$), 138.7 (C_1), 133.4, 128.5, 121.0, 108.4, 108.1 ($C_{2,5,6,8,9}$), 101.0 (C_{22}), 47.2, 46.8, 36.6, 35.6 ($C_{7,10,15,16}$) 46.4, 45.4 ($C_{18,21}$), 30.6, 28.8, 26.4, 26.0, 24.3 and 22.1 ($C_{11,12,13,14,19,20}$). The off-resonance ^{13}C-NMR-spectrum excluded the presence of quaternary sp^3-carbons in the alkaloid.

[1] For Part I see B. S. JOSHI, N. VISWANATHAN, D. H. GAWAD and W. VON PHILIPSBORN, Helv. chim. Acta, in preparation.

[2] Contribution No. 402 from Ciba-Geigy Research Centre; ^{13}C-NMR-spectroscopy Part 8, for Part 7 see Ref.[1].